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Reducing odorous VOC emissions from swine manure using soybean peroxidase and peroxides

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HIGHLIGHTS

- Soybean peroxidase and peroxides were applied to swine manure for odor control.
- The optimum industrial-grade soybean peroxidase rate was 50 g L^{-1}.
- Calcium peroxide was more effective than hydrogen peroxide.
- The primary odorant 4-methylphenol accounted for 68–81% of the odor activity value.
- A 4-methylphenol reduction of 92% was achieved.

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ABSTRACT

The objective of the research was to determine the optimum application rates of soybean peroxidase (SBP) plus peroxide (SBPP) for reducing odorous VOC emissions from swine manure. Industrial-grade SBP was applied in combination with liquid hydrogen peroxide (H_2O_2) or powdered calcium peroxide (CaO_2) to standard phenolic solutions and swine manure, and emissions were measured in a wind tunnel. The primary odorant in the untreated manure was 4-methylphenol, which accounted for 68–81% of the odor activity value. At the optimum application rate of SBPP (50 g L^{-1}), 4-methylphenol emissions were reduced from the swine manure by 62% (H_2O_2) and 98% (CaO_2) after 24 h ($P < 0.0001$). The CaO_2 had a longer residence time, remaining effective for 48 h with 92% reduction in emission rates ($P < 0.0001$), while H_2O_2 was similar to the control at 48 h ($P = 0.28$).

1. Introduction

Volatile organic compounds (VOC) are emitted from wastewater treatment plants, food processing plants, landfills, and animal feeding operations (Kim et al., 2006). Volatile organic compound emissions are an important aspect of air quality because many are malodorous. There has been a growing concern in recent years over VOC emissions and the related nuisances from agricultural operations, prompting several states to enact ambient odor regulations (Sweeten, 1995; Chen et al., 1999; CAQCC, 1999; Redwine and Lacey, 2000).

More than 200 VOC have been identified in air emissions from manure and animal feeding operations (Kai and Schafer, 2004; Akdeniz et al., 2010). These VOC originate from the degradation of amino acids in the intestines of animals and anaerobic decomposition of manure (Mata-Alvarez et al., 2000; Kai and Schafer, 2004; Chen et al., 2008). Ammonia, volatile fatty acids (VFA), sulfides, 4-methylphenol (i.e. *p*-cresol), phenol, indole, and skatole are among the most commonly reported odorants associated with animal manures (Mackie et al., 1998). Many odorous compounds are emitted at animal feeding operations, but it is the phenolic and indolic compounds that dominate the overall odor at distance from these operations (Parker, 2007). As such, these compounds are the

Abbreviations: SBP, soybean peroxidase; SBPP, soybean peroxidase plus peroxide; HRP, horseradish peroxidase; VFA, volatile fatty acid; VOC, volatile organic compound; TD, thermal desorption; GC, gas chromatography; MS, mass spectrometry; DM, dry matter; OAV, odor activity value.

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logical target for reduction of specific odorous VOCs in areas affected by animal feeding activities.

Peroxidase enzymes have been investigated for use in the abatement of phenolic contaminants in wastewaters (Morawski et al., 2001). In the presence of peroxides such as hydrogen peroxide (H_2O_2) or calcium peroxide (CaO_2), the peroxidase polymerizes some phenolic and indolic compounds, making them insoluble and non-volatile compounds and thereby reducing the odor (Tonegawa et al., 2003). There are many different kinds of peroxidase enzymes, but horseradish peroxidase (HRP) and soybean peroxidase (SBP) have been the most studied. HRP and SBP are naturally occurring enzymes isolated from plant roots and above ground biomass. The medicinal and enzymatic properties of HRP have been studied for more than two centuries (Veitch, 2004).

While there has been considerable research on peroxidase enzymes for treatment of wastewaters, only recently was the technology applied to treatment of manure for odor control. Govere et al. (2007) demonstrated the removal of phenolic compounds from swine manure using minced horseradish roots, while Ye et al. (2009) showed that purified HRP was efficient at reducing concentrations of phenol, 4-methylphenol, indole, and skatole in diethyl ether extracts taken from swine manure slurry. The primary drawback to using raw horseradish root is the limited supply, while the drawback of purified HRP is the excessive cost. The highest activity of SBP is found in the soybean hulls (i.e. the seed coat) of the plant (Gijzen et al., 1993). Soybean hulls are a relatively inexpensive byproduct of soybean processing for oil and meal production. Soybean peroxidase and particularly soybean hulls are more plentiful than HRP because soybeans are one of the most abundant crops produced in the world. The U.S. produced about 81 million metric tons of soybeans on 30 million hectares in 2007 (Masuda and Goldsmith, 2009). In contrast, only about 10,000 metric tons of horseradish are harvested from 2000 hectares in the U.S. annually (Bratsch, 2009).

The initial objectives of this research were to evaluate and optimize a commercially-available plant-derived SBP enzyme product for reducing odorous VOC emissions from swine manure. In order to optimize its application toward odor reduction, we further compared the efficacy of SBP in combination with one of two peroxide sources (liquid hydrogen peroxide and powdered calcium peroxide) in a method named SBPP for soybean peroxidase plus peroxide.

2. Methods

2.1. Experimental design

A total of 12 experiments were conducted, and the five experiments that best describe the treatment system are presented in this manuscript. The first two experiments employed standard solutions of phenol and 4-methylphenol while the last three employed actual slurries of swine manure (Table 1). Experiment 1 varied the concentration of SBP with a constant level of H_2O_2, while Experiment 2 varied the concentration of H_2O_2 with a constant level of SBP. In Experiments 3–5, treatments were applied to swine manure slurry. For all experiments, 200 mL of standard solution or swine manure slurry were placed in 500 mL glass beakers where the treatments were applied. Following addition of SBP and either H_2O_2 or CaO_2, the components were thoroughly mixed. The mixtures were incubated in the beakers for 2–72 h, and then 175 mL of the standard solution or 175 g of the manure solution was transferred to a 138 mm diameter Petri dish to measure VOC emissions in a small wind tunnel. Further details on individual experiments are provided below and in Table 1.

2.1.1. Experiment 1

Experiment 1 was conducted to evaluate the effect of varying SBP application rate when applied to a standard phenolic solution consisting of 1 mM phenol and 1 mM 4-methylphenol in distilled water. There was a control treatment (no additives) and SBP treatments of 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 g L^{-1} each with 2.64 mM H_2O_2. The SBP and H_2O_2 were thoroughly mixed in a glass beaker containing the phenolic standard solution and incubated at 21.0 °C. After 2 h, the treated solutions were transferred to Petri dishes, and emissions of phenol and 4-methylphenol were measured in a small wind tunnel. For this experiment and the subsequent experiments, there was one Petri dish per treatment, and two sorbent tube VOC samples were collected on each Petri dish.

2.1.2. Experiment 2

Experiment 2 was conducted to evaluate the effect of varying H_2O_2 concentrations when applied to the same standard phenolic solution containing 1 mM phenol and 1 mM 4-methylphenol in distilled water. There was a control treatment (no additives) and H_2O_2 treatments of 0.4, 1.3, 2.6, 5.3, 10.6, and 44.1 mM. Each H_2O_2 treatment also received 5 g L^{-1} SBP. Phenol and 4-methylphenol emissions were measured after 2 h.

2.1.3. Experiment 3

Experiment 3 was conducted to optimize SBP concentrations when applied with H_2O_2 to swine manure slurry. There was a control treatment (no additives) and SBP dosages of 5, 25, 50, 75, and 100 g L^{-1}. All SBP treatments were accompanied by H_2O_2 at concentrations of 14.6 mM. Volatile organic compound emissions were measured at times of 2, 24, 48, and 72 h after treatment.

2.1.4. Experiment 4

Experiment 4 was conducted to optimize H_2O_2 or CaO_2 concentrations when applied with SBP to swine manure. There were six manure treatments that all received the fixed amount of SBP at 50 g L^{-1}, and a control treatment that received neither SBP nor peroxide. Two of the SBP treatments received H_2O_2 at concentrations of 14.6 and 38.0 mM. Four of the SBP treatments received CaO_2 at concentrations of 14.6, 22.1, 38.0, and 76.0 mM. Volatile organic compound emissions were measured at times of 2, 24, and 48 h after treatment.

Table 1
Details of the five soybean peroxidase plus peroxide (SBPP) experiments designed to optimize the dosages of soybean peroxidase (SBP) with either hydrogen peroxide (H_2O_2) or calcium peroxide (CaO_2).

Experiment No.	Purpose	Source	Time evaluated (h)	SBP added (g/L)	H_2O_2 (mM)	CaO_2 (mM)
1	Optimize SBP	Std. solution[a]	2	0–10	2.65	0
2	Optimize H_2O_2	Std. solution	2	5	0–44.1	0
3	Optimize SBP	Swine manure	2, 24, 48, 72	0–100	14.6	0
4	Optimize H_2O_2 and CaO_2	Swine manure	2, 24, 48	50	0–38.0	0–76.0
5	Replicated experiment	Swine manure	2, 24, 48	50	14.6	22.1

[a] Std. solution of 1 mM phenol and 1 mM 4-methylphenol.

2.1.5. Experiment 5

A final replicated experiment was conducted to compare the most effective treatments from Experiments 3 and 4. The three treatments consisted of (1) a control with no amendments, (2) 14.6 mM H_2O_2 plus 50 g L^{-1} SBP, (3) 22.1 mM CaO_2 plus 50 m L^{-1} SBP. There were three replicates of each treatment, and emissions were measured at times of 2, 24, and 48 h following application. In addition, pH of swine slurry was measured at times of 0, 2, 24, and 48 h.

2.2. Reagents, soybean peroxidase, and swine manure

The SBP used in this study was an industrial grade product (Cat. No. X516-IND; Bio-Research Products Inc., North Liberty, Iowa, USA) which had a tan-reddish fine powder with a nominal activity of 0.77 U mg^{-1} (U = guaiacol activity units). Liquid hydrogen peroxide (30%, ACS grade), powdered calcium peroxide (20%, ACS grade), and phenol and 4-methylphenol (>99%, ACS grade) were obtained from Fisher Scientific (St. Louis, Missouri, USA).

Swine manure was collected from swine barns at the USDA-ARS U.S. Meat Animal Research Center in Clay Center, Nebraska, USA. The pigs were fed a corn-based diet with soybean meal (80.2% ground corn, 16.6% soybean meal with the remainder being supplemental vitamins, minerals, amino acids, and antibiotics). The manure was mixed with equal mass of distilled water to form a pourable slurry, and was transferred to individual glass beakers where the treatments were applied. The swine manure slurry had total solids contents (dry matter, DM) of 13.2%, and the volatile solids content was 20.0% of DM (2.6% ash).

2.3. VOC concentration and flux measurement

Volatile organic compound flux was measured in a small rectangular wind tunnel of 51 mm height, 305 mm length, and 152 mm width with a 5 mm thick Plexiglas top. The wind tunnel has been described previously (Parker et al., in press). Sweep air entered the wind tunnel through 17 holes (6-mm dia.) in three rows at heights of 17 mm (6 holes), 30 mm (5 holes), and 43 mm (6 holes) above the base. Air exited the tunnel through three 10-mm diameter holes equally spaced at height 27 mm above the base at the opposite end of the tunnel. As the sweep air was passed through activated carbon and Drierite™ filters to remove VOCs and moisture, it was verified as VOC free. The sweep air flow rate was controlled using a valved rotameter with scale of 0–1.2 L min^{-1} (Omega Engineering, Stamford, CT, USA).

A round Petri dish (138 mm dia × 13 mm, 200 mL) containing the swine slurry sample was placed in the center of the wind tunnel. The wind tunnel was operated at a sweep air flow rate of 1 L min^{-1}, corresponding to sweep air volumetric exchange rate of 0.4 exchanges min^{-1}. At this air flow rate, the equivalent distilled water evaporation rate, measured using dry sweep air with the same size Petri dish at 21.0 °C, was 1.2 mm d^{-1}.

A fraction of the air exiting the wind tunnel was sampled using stainless steel sorbent tubes (89 mm × 6.4 mm OD) packed with 200 mg Tenax TA® sorbent. Conditioned and capped tubes were purchased new from the manufacturer (Markes International Inc., Wilmington, DE, USA). Prior to first use, tubes were conditioned for 2 h at 230 °C and verified to be clean before sampling. Air was pulled through the sorbent tubes at a flow rate of 75 mL min^{-1} for 2 min for a sample volume of 150 mL using a vacuum pump (Pocket pump 210 series, SKC Inc., Eighty Four, PA, USA). All sampling was conducted at room temperature of 21.0 °C.

2.4. GC/MS with thermal desorption

Sorbent tube samples were analyzed using a thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS) system. The TD system consisted of a Markes Unity 2 thermal desorber with Ultra 2 autosampler (Markes International Inc., Wilmington, DE, USA). Samples were quantified with an Agilent 7890A GC with Agilent 5975C MS (Agilent Technologies, Inc., Santa Clara, CA, USA).

In the TD system, samples were initially purged for 2 min (40 mL min^{-1}, 20 °C) to remove water and air. The tube was desorbed for 10 min at 280 °C with a carrier gas flow of 50 mL min^{-1} and trapped on the cold trap maintained at −10 °C. The cold trap was heated to 320 °C for 3 min with a carrier gas flow of 20, and 1.4 mL min^{-1} was transferred to the column in the GC–MS. In the GC oven, the column was held at 40 °C for 3 min, ramped to 230 °C at 8 °C min^{-1}, then held at 230 °C for 5 min for a total run time of 31.8 min. The polar analytical column was an Agilent Innowax, 30 m × 0.25 mm ID capillary column (polyethylene glycol, 0.25 μm film thickness).

2.5. Standards and calibration

Samples were analyzed for VFAs, phenolics, and indolics. Standard solutions were prepared by diluting known masses of pure chemicals with methanol. Standards were prepared using serial dilutions, and injected onto clean tubes using a calibration solution loading rig (CSLR; Markes International Inc., Wilmington, DE, USA). The liquid calibration standard was introduced through the CSLR injector septum in clean bottled air carrier gas (75 mL min^{-1}) using a standard GC syringe. Within the linear range, standard curves were fit using linear regression with the curve forced through the origin.

All sorbent tubes were conditioned with the TD system and analyzed on the GC–MS prior to sampling to verify that clean tubes were used in the experiment. Regression slopes and r^2 values for each compound are shown in Table 2.

2.6. Statistical analysis

Data from Experiments 1 to 4 were graphed and visually examined to determine the optimum application rates of HRP, SBP, and peroxides. The data from Experiment 5 were analyzed as a completely randomized design with repeated measures in time using the SAS MIXED Procedure (SAS Inst. Inc., Cary, NC). Fixed effects were treatment, replicate, time, and the treatment × time interaction. Replicate × treatment was the subject of the repeated measures, and several covariance structures were tested. The covariance structure resulting in the smallest Akaike and Schwarz Bayesian criteria were considered most appropriate for analysis. When significant ($P < 0.05$) treatment × time interactions were detected using single-degree-of-freedom F-tests, pairwise comparisons of the simple-effects means were conducted within the PDIFF option of SAS. Effects were considered significant at $P < 0.05$.

2.7. Odor activity value

Odor activity values (OAV), defined as the ratio of the concentration of a compound to the human odor threshold for that compound (Guadagni et al., 1963; Leffingwell and Leffingwell, 1991; Parker et al., in press), were calculated to assess the importance of individual chemical compound reductions (or increases) on odor. Compounds with large OAV are more likely to contribute to the overall odor of a complex odor mixture. Odor activity values were calculated using published odor thresholds for each compound (Parker et al., in press). The relative contribution of each compound was calculated by dividing the OAV for the individual compound by the sum of the OAVs for all compounds (Guadagni et al., 1963; Leffingwell and Leffingwell, 1991).

Table 2
Summary of compounds quantified by GC/MS in the study, linear range and regression statistics for standard curves.

Compound	MW[a]	Retention time (min)	MS quan ion[a]	Linear range (ng)	Linear regression slope (B1)	Linear regression (r^2)
Phenol	94.1	20.2	94	5.7–1105	1.31E−04	0.992
4-Methylphenol	108.1	21.1	107	5.2–1045	1.37E−04	0.973
Indole	117.1	25.2	117	6.3–1283	6.60E−05	0.988
Skatole	131.2	25.6	130	7.1–722	4.30E−05	0.969
Acetic acid	60.0	12.4	60	2.6–2602	8.18E−04	0.997
Propanoic acid	74.1	13.8	74	2.5–2485	7.58E−04	0.999
Isobutyric acid	88.1	14.2	43	2.3–2344	4.53E−04	0.997
Butyric acid	88.1	15.1	60	2.4–2401	2.94E−04	0.995
Isovaleric acid	102.1	15.7	60	2.3–2290	2.69E−04	0.991
Valeric acid	102.1	16.7	60	2.3–2342	2.51E−04	0.990
Hexanoic acid	116.2	18.1	60	2.3–2348	2.97E−04	0.978
Heptanoic acid	130.2	19.5	60	2.3–2278	3.57E−04	0.982

[a] MW = molecular weight, MS = mass spectrometry quantification ion.

3. Results and discussion

3.1. Optimization of SBPP when applied to standard solutions (Experiment 1)

Experiment 1 varied the concentration of SBP with a constant level of H_2O_2. Although the flux of phenol and 4-methylphenol from the standard solution both decreased with increasing SBP application rate, 4-methylphenol was more readily treated than phenol (Fig. 1a). There was a 96.5% reduction in 4-methylphenol flux with SBP application rate of around 1.0 g L^{-1}. Reductions of 4-methylphenol were 99.9%, 99.8%, and 96.9% at SBP application rates of 2.5, 5.0, and 10.0 g L^{-1}, respectively. Thus, increasing the SBP application rate from 1.0 to 2.5 g L^{-1} only improved the reduction by an additional 3.4%. However, in terms of potential odor emissions, the 2.5 g L^{-1} application rate would be recommended. This is because the geometric mean odor threshold of 1.3 μg m^{-3} for 4-methylphenol (Parker et al., in press) equates to a flux of 0.09 μg m^{-2} min^{-1} from the wind tunnel, and 4-methylphenol fluxes were 7.28 and 0.22 μg m^{-2} min^{-1} at SBP application rates of 1.0 and 2.5 g L^{-1}, respectively. Thus, the SBP rate of 2.5 g L^{-1} reduced the 4-methylphenol flux almost to the odor threshold. This feature is important because at this concentration humans would no longer be able to smell the 4-methylphenol. The effectiveness of 4-methylphenol reduction decreased slightly at the highest SBP application rate of 10.0 g L^{-1}. It is unknown whether this is a direct effect of higher SBP application rate, or simply an artifact of experimental variation.

The geometric mean odor threshold for phenol (206 μg m^{-3}) is more than two orders of magnitude greater than that for 4-methylphenol (Parker et al., in press), and consequently 4-methylphenol is often implicated as the most important odorous VOC at animal feeding operations (Parker, 2007). A majority of the urinary phenols excreted by livestock are 4-methylphenol and, as the dietary protein increases, 4-methylphenol excretion increases as a result of microbial amino acid fermentation in the lower gut (Mackie et al., 1998). In recent years, cattle and swine diets contain increased levels of distillers grains and soluble (DGS), a byproduct of the growing ethanol industry. There is approximately three times more protein in DGS than in corn. The fact that SBP selectively treats 4-methylphenol faster than phenol (Fig. 1a) is a tremendous advantage for odor reduction.

For phenol, the flux decreased almost linearly for SBP application rates from 0 to 5.0 g L^{-1} (Fig. 1a) but there were minimal differences in percent reduction between SBP application rates of 5.0 and 10.0 g L^{-1} (86.9% and 84.0% reductions, respectively). At the SBP application rate of 2.5 g L^{-1}, there was a 65% reduction in the phenol emission rate in addition to the 99.9% reduction in 4-methylphenol. With an SBP nominal activity of 0.77 U mg^{-1}, the SBP application rate of 2.5 g L^{-1} was equivalent to 1925 U L^{-1} (2.5 g L^{-1} × 1000 mg g^{-1} × 0.77 U mg^{-1}). Caza et al. (1999) reported a 95% reduction in phenolic substrate when treated with 800 U L^{-1} of SBP. Given that we had two phenolic substrates (phenol and 4-methylphenol), then Caza's recommendation of 800 U L^{-1} would equate to 1600 U L^{-1}, which compares favorably to the 1925 U L^{-1} in our research. Flock et al. (1999) measured a 60% reduction in phenol solution (with 1000 ppm initial concentration) with the addition of 40 g L^{-1} fresh soybean hulls.

3.2. Optimization of H_2O_2 when applied to standard solutions (Experiment 2)

Experiment 2 varied the concentration of H_2O_2 with a constant level of SBP. There were considerable differences in the treatment

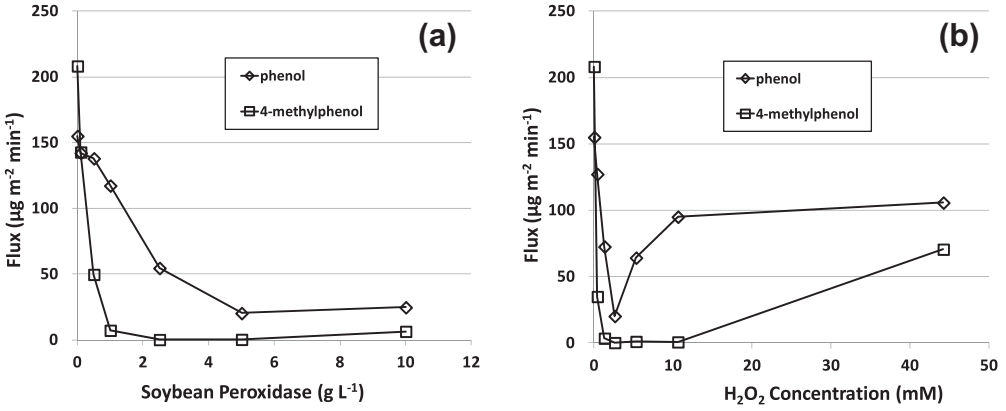

Fig. 1. Flux from standard solution of 1 mM phenol and 1 mM 4-methylphenol 2 h after treatment as affected by (a) varying soybean peroxidase with constant 2.64 mM H_2O_2, and (b) varying H_2O_2 concentration with constant 5 g L^{-1} SBP (Experiments 1 and 2).

efficacies of phenol and 4-methylphenol at different H_2O_2 concentrations (Fig. 1b). While the fluxes of both compounds decreased quickly between H_2O_2 concentrations of 0 and 2.6 mM, the phenol flux actually increased above 2.4 mM. For the phenol treatment there was a narrow window of effective H_2O_2 concentration, while for 4-methylphenol the window was much broader. The 4-methylphenol flux stayed low up to 10.6 mM, with percent reductions of 98.3% to 98.6% between 1.3 and 10.6 mM, respectively (Fig. 1b). As stated previously, the odor threshold for 4-methylphenol is about two orders of magnitude lower than that for phenol. Thus, this broad window of effective H_2O_2 concentration for 4-methylphenol would be desirable for odor reduction. The 4-methylphenol flux was at a minimum (0.34 μg m^{-2} min^{-1}) at an H_2O_2 concentration of 2.6 mM. Thus, for the standard solution of phenol and 4-methylphenol, the optimum H_2O_2 concentration was 2.6 mM, although differences were minimal between 1.3 and 10.6 mM H_2O_2.

The optimum H_2O_2 concentration of 2.6 mM for the sum of both phenolic compounds compares very favorably with Caza et al. (1999), who reported optimum H_2O_2 concentrations of 1.2 per mM of phenol and 0.9 per mM 4-methylphenol (sum of 2.1 mM).

3.3. Optimization of SBPP when applied to swine manure (Experiment 3)

Experiment 3 varied the concentration of SBP with a constant level of H_2O_2. The concentrations of phenol and 4-methylphenol in the manure slurry were 0.065 and 0.267 mM, respectively. Even though these concentrations were considerably less than the 1 mM for each compound in the standard solution, it took more than 10 times the SBP for optimal treatment in the manure than in the standard solution. While the exact cause for this increased SBP requirement is unknown, it is likely that there were various interfering constituents in the manure but not the standard solution.

For untreated swine manure, the flux of 4-methylphenol (range 31–46 μg m^{-2} min^{-1}) was considerably greater than for the other VOCs analyzed (Fig. 2). The flux of 4-methylphenol decreased with increasing SBP rate and the SBP was effective in treating 4-methylphenol for the full 72 h duration of the experiment. The fluxes of indole and skatole decreased by 30–50%. Indole is the product of tryptophan metabolism (Macfarlane and Macfarlane, 1995) and it has been reported to increase in swine feces when feeding dried distillers grains (Hawe et al., 1992), which again demonstrates the importance of SBP in abatement of odor in swine manure.

An interesting aspect of these results was that the lowest SBP rate of 5.0 g L^{-1} actually increased 4-methylphenol emissions up to 50% over the controls (Fig. 2). The cause in this bump, which had disappeared by 25 g L^{-1} (Fig. 2), is still unknown but it could be because the dry SBP preferentially absorbs water but not VOCs, leaving higher concentrations of VOC in solution. A similar phenomenon was observed by the authors in experiments involving the treatment of manure with zeolite (Donnell et al., 2008).

Two hours after treatment, there was a 69.1% reduction in 4-methylphenol at SBP application rate of 50 g L^{-1}. Since there was little difference in 4-methylphenol flux between the 50, 75, and 100 g L^{-1} SBP application rates after 72 h (Fig. 2), the SBP application rate of 50 g L^{-1} was concluded to be the optimum for swine manure.

3.4. Optimization of H_2O_2 or CaO_2 when applied to swine manure (Experiment 4)

Experiment 4 compared concentrations of H_2O_2 and CaO_2 with a constant level of SBP. While it only required 2.64 mM H_2O_2 to treat the 2 mM phenolic standard solution (Fig. 1b), it took approximately five to nine times more peroxide for the manure (Fig. 2).

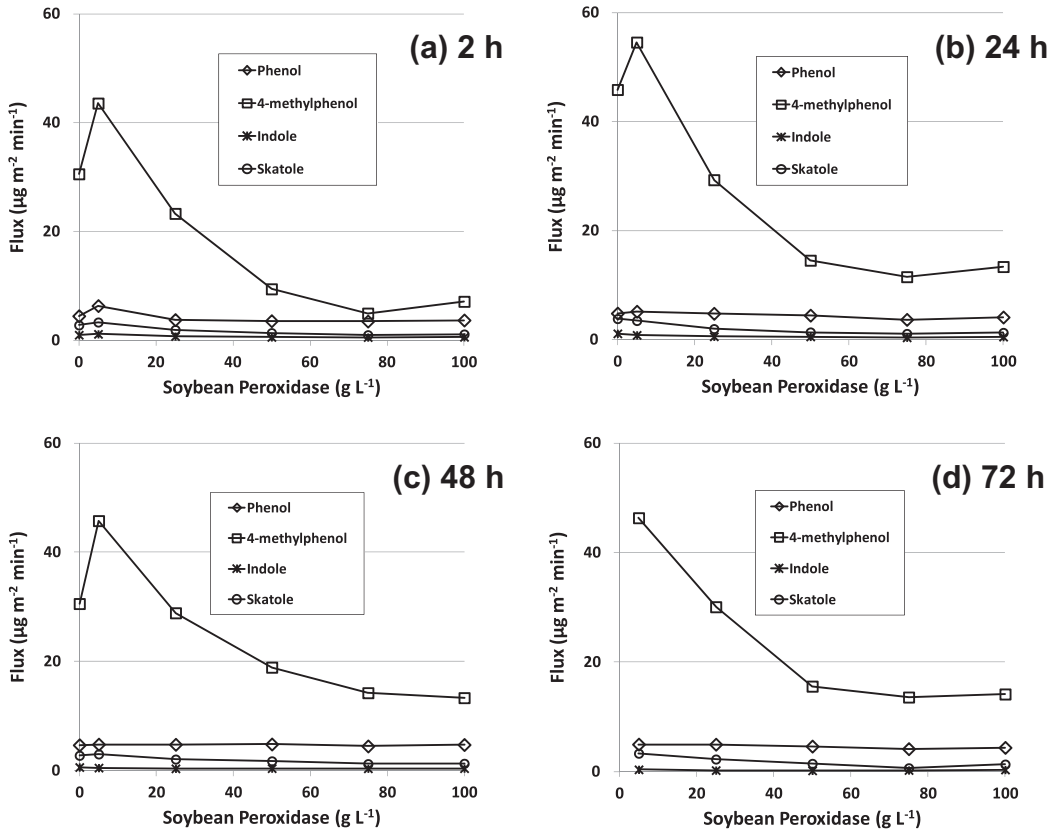

Fig. 2. Flux of phenolic and indolic compounds from swine slurry at times of 2, 24, 48, and 72 h after addition of SBP (Experiment 3). Each treatment except for the control received SBP plus 14.6 mM H_2O_2.

Table 3
Flux of phenolic and indolic compounds over time as affected by type and amount of peroxide (Experiment 4). All treatments except for the control received 50 g L^{-1} soybean peroxidase (SBP) plus peroxide in the amount shown.

	Control	H_2O_2 14.6 mM	H_2O_2 38.0 mM	CaO_2 14.6 mM	CaO_2 22.1 mM	CaO_2 38.0 mM	CaO_2 76.0 mM
Flux after 2 h ($\mu g\ m^{-2}\ min^{-1}$)							
Phenol	3.97	3.60	4.20	3.74	3.45	3.20	1.80
4-Methylphenol	24.64	11.1	8.80	9.44	2.43	5.85	2.03
Indole	9.63	0.50	0.50	0.96	1.31	1.51	2.21
Skatole	10.21	1.0	1.2	1.61	1.77	2.67	5.13
Flux after 24 h ($\mu g\ m^{-2}\ min^{-1}$)							
Phenol	5.19	4.11	4.23	2.98	2.20	1.37	1.19
4-Methylphenol	55.86	18.72	13.65	3.26	0.82	2.11	1.30
Indole	20.91	0.74	0.62	1.19	1.31	1.18	2.02
Skatole	22.71	1.98	1.85	1.76	1.76	2.03	4.25
Flux after 48 h ($\mu g\ m^{-2}\ min^{-1}$)							
Phenol	5.91	4.15	3.77	3.70	2.11	2.05	1.03
4-Methylphenol	58.35	18.49	13.56	3.15	1.11	4.33	1.62
Indole	22.29	0.38	0.40	0.91	1.13	1.32	2.09
Skatole	24.98	1.85	1.64	1.39	1.44	2.40	4.57

This increase was likely because some of the peroxide was inactivated by the organic matter in the manure, making it unavailable for oxidation in the peroxidase enzyme process.

Experiment 4 further demonstrated this limitation of liquid H_2O_2, especially in contrast to the alternative solid CaO_2 (Table 3). Two hours after treatment, there was little difference in 4-methylphenol reductions between the 14.6 mM CaO_2 and H_2O_2 treatments (61.7% and 55.0%, respectively). However, after 24 h the CaO_2 treatment performed considerably better than did H_2O_2, with 4-methylphenol reductions of 94.2% and 66.5%, respectively. For 4-methylphenol, the CaO_2 treatments generally outperformed the H_2O_2 treatments by about 20–30%.

Percent reductions in phenol were minimal after 2 h, but after 24 and 48 h all treatments became more effective with percent reductions of 30–80%. As for 4-methylphenol, the CaO_2 treatments performed better than the H_2O_2 treatments. Percent reduction in phenol flux was positively correlated with CaO_2 concentration at 24 and 48 h, with 82.5% reduction in phenol flux at the highest CaO_2 concentration of 76.0 mM. Both peroxides were effective at reducing indole and skatole flux, with percent reductions of about 80–98% (Table 3). Overall, based on the 4-methylphenol reductions, we concluded that the optimum H_2O_2 and CaO_2 concentrations were 14.6 and 22.1 mM, respectively.

While CaO_2 outperformed H_2O_2 for the reduction of phenolic and indolic compounds, another potential benefit of CaO_2 is that it is a dry powder, similar to the SBP. Should SBP become a potential additive to swine barns or lagoons, the potential exists for mixing the two powders (SBP and CaO_2) in dry form for topical application. Future research will focus on topical application in laboratory and field experiments.

3.5. Replicated experiment (Experiment 5)

The optimum H_2O_2 and CaO_2 concentrations (Table 3) were confirmed by Experiment 5 in which the data for each time point were obtained in triplicate. The treatment × time interactions were significant ($P < 0.05$) for all compounds. Therefore, only the treatment × time means were statistically compared (Table 4). At the optimum SBP application rate for swine manure of 50 g L^{-1} (as determined in Fig. 2), emissions of the primary odorant 4-methylphenol were reduced by 62% and 98% ($P < 0.0001$) after 24 h for H_2O_2 and CaO_2, respectively. After 48 h, the CaO_2 treatment remained effective with 92% reduction ($P < 0.0001$), but there was no difference between the H_2O_2 treatment and the control ($P = 0.28$) (Fig. 3b). However, neither of the SBP treatments were effective at reducing phenol emissions (Fig. 3a).

After 24 h, SBP was effective at reducing indole concentrations by 60% ($P < 0.0001$) for H_2O_2 and 19% ($P = 0.039$) for CaO_2 but after 48 h, the CaO_2 treatment actually increased indole emissions by 69% ($P < 0.0001$) while the H_2O_2 treatment was again

Table 4
Least squares means ($\mu g\ m^{-2}\ min^{-1}$) for the control with no additives (TRT 1), 50 g L^{-1} soybean peroxidase (SBP) plus 14.5 mM H_2O_2 (TRT 2), and 50 g L^{-1} SBP plus 22.1 mM CaO_2 (TRT 3) of Experiment 5.

	2 h			24 h			48 h		
	Control (TRT 1)	H_2O_2 (TRT 2)	CaO_2 (TRT 3)	Control (TRT 1)	H_2O_2 (TRT 2)	CaO_2 (TRT 3)	Control (TRT 1)	H_2O_2 (TRT 2)	CaO_2 (TRT 3)
Phenol	2.82 a	2.55 a	2.59 a	3.72 f	3.34 f	2.16g	3.04 y	3.80 x	3.32 xy
4-Methylphenol	39.20 a	14.00 b	7.20 c	75.83 f	28.47 g	1.23 h	23.93 x	20.47 x	1.97 y
Indole	0.83 b	0.50 c	0.99 a	1.10 f	0.44 h	0.89 g	0.24 y	0.22 y	0.77 x
Skatole	2.21 a	1.22 b	2.26 a	5.20 f	2.25 g	1.73 h	1.22 y	1.59 xy	1.84 x
Acetic Acid	38.92 a	37.85 a	17.54 a	68.30 f	60.61 f	15.04 f	31.79 y	283.80 x	53.72 y
Propionic Acid	27.05 a	29.32 a	2.59 b	77.35 f	70.79 f	2.76 g	20.25 y	260.93 x	13.08 y
Isobutyric Acid	5.25 a	6.92 a	0.74 b	14.22 f	14.41 f	0.79 g	3.90 y	50.68 x	2.54 y
Butyric Acid	17.73 a	18.21 a	1.31 b	60.67 f	57.66 f	1.34 g	11.13 y	238.17 x	5.76 y
Isovaleric Acid	3.17 a	3.22 a	0.34 b	11.15 f	11.07 f	0.31 g	2.15 y	50.50 x	1.38 y
Valeric Acid	2.94 a	2.34 a	0.62 a	9.98 f	10.96 f	0.80 g	2.09 y	64.78 x	2.21 y
Hexanoic Acid	0.80 a	0.58 a	0.65 a	0.99 f	1.02 f	0.34 f	0.33 y	5.14 x	0.76 y
Heptanoic Acid	0.38 a	0.41 a	0.12 b	0.29 g	0.63 f	0.12 g	0.30 y	0.88 x	0.39 y
Total VFAs	96.24 ab	98.85 a	23.91b	242.9 f	227.2 f	21.50 g	82.24 y	954.9 x	79.83 y
Manure pH (pH units)	6.00 b	6.00 b	9.00 a	5.80 h	5.90 g	9.17 f	5.80 y	5.07 z	6.20 x

a–c Means within a row for a specific time period (2 h) with different letters differ ($P \leqslant 0.05$).
f–h Means within a row for a specific time period (24 h) with different letters differ ($P \leqslant 0.05$).
x–z Means within a row for a specific time period (48 h) with different letters differ ($P \leqslant 0.05$).

indistinguishable from the control ($P = 0.73$). Skatole emissions were significantly reduced with both peroxide treatments at 24 h ($P < 0.0001$), but at 48 h the CaO_2 treatment was 51% greater than the control ($P = 0.005$).

The VFAs were dominated by acetic acid, propionic acid, and butyric acid, which comprised from 77% to 91% of the total VFAs (Table 4). Similar percentages in swine manure have been reported by others (Velthof et al., 2005; Conn et al., 2007). The CaO_2 treatment reduced the total VFA flux by 75% at 2 h ($P = 0.053$) and 91% at 24 h ($P < 0.0001$) (Fig. 3e). However, after 48 h there was no difference between VFA flux for the control and CaO_2 treatments ($P = 0.90$). More remarkably, after 48 h the H_2O_2 treatment increased total VFA emissions by 1060% (more than 10-fold, $P < 0.0001$). This phenomenon is most likely the result of manure pH. The manure used in this research had low initial pH, but still within the range of expected manure pH values for anaerobic manure. In laboratory studies, Miller and Varel (2003) reported pH values of 6.1–5.4 during the incubation of swine manure slurry. As compared to the control (pH = 5.8), there was also a decrease in manure pH of 0.7 units for the H_2O_2 treatment (pH = 5.1) after 48 h. Volatilization of VFAs has been shown to be highly dependent on manure pH (Miller and Berry, 2005). The pK_a values for VFAs are approximately 4.8 and at low pH values the VFAs exist in the volatile non-ionized form, whereas at higher pH values they exist in the non-volatile ionized form. Thus, as the pH for the H_2O_2 treated manure neared the pK_a for VFAs there was a considerable increase in VFA emissions (Table 4). In contrast, CaO_2 treatment increased the manure pH at 2 and 24 h by about three pH units (pH > 9), considerably above the pK_a values for VFAs, and at 48 h the pH was still significantly greater than the starting pH (Table 4). As

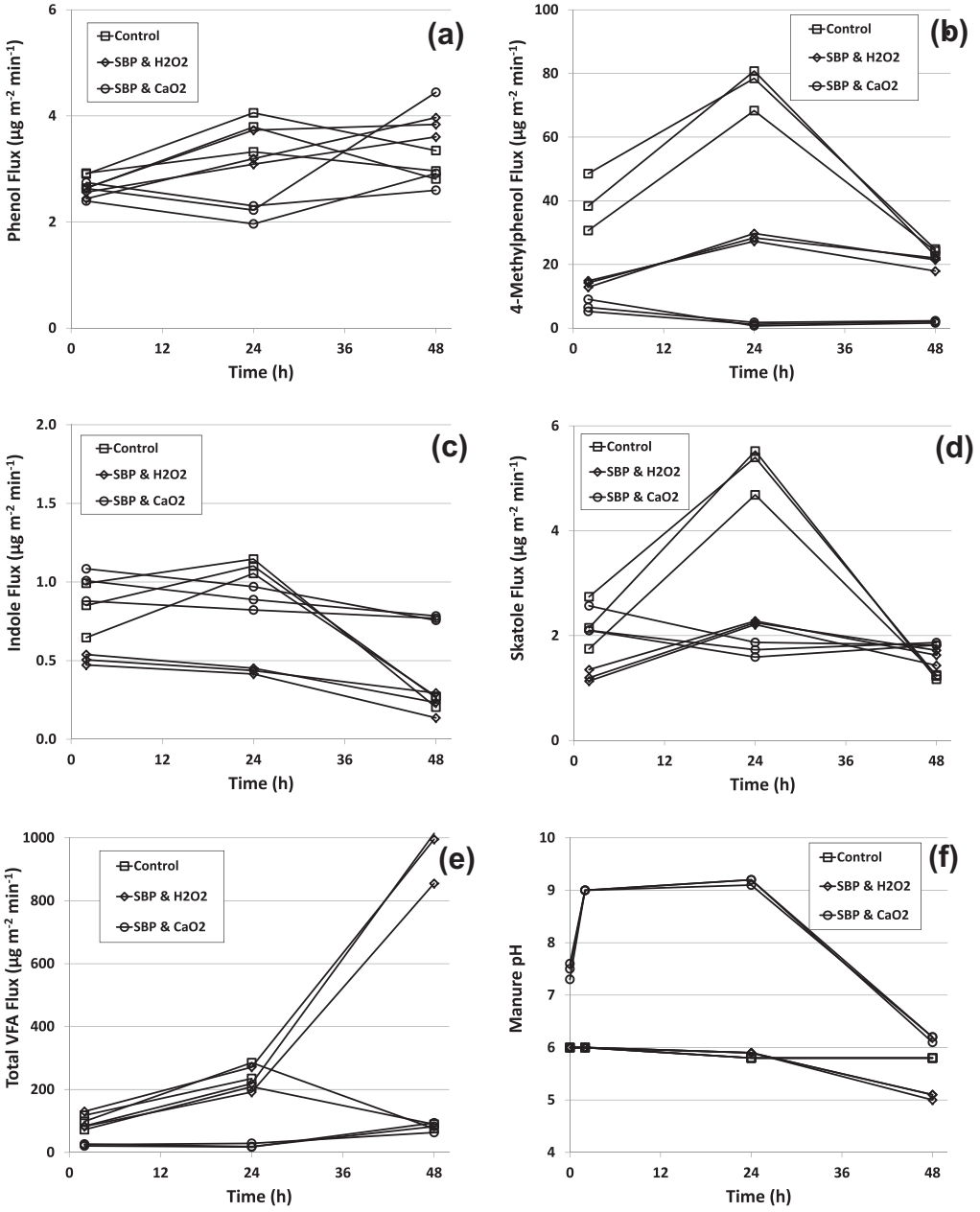

Fig. 3. Variation of phenolic, indolic, volatile fatty acid flux, and manure pH over time for control (TRT 1, no additives), SBP plus H_2O_2 (TRT 2), and SBP plus CaO_2 (TRT 3) from Experiment 5.

an alternative explanation for the greatly increased VFAs observed (Table 4), the H_2O_2 could steer microbial fermentation end products from amino acid derived alcohols and aldehydes to the more oxidized acids (Hazelwood et al., 2008). These two possible explanations are fully compatible with one another.

When conducting the emission measurements the researchers observed considerable differences in odors between the control and treated manure samples. The control smelled like typical swine manure, yet the SBP/CaO_2 treated manure was described by the researchers as being considerably less offensive and having 'earthy' and 'grainy' character notes. One researcher described the SBP/CaO_2 treated manure as smelling like 'cooked oatmeal'. Significantly less microbial activity would be expected at pH 9 than at pH 5.1–6.0 (Table 4).

3.6. Effectiveness of 4-methylphenol reduction for odor control in swine manure

While the SBPP treatments caused a considerable decrease in 4-methylphenol emissions, the emissions of some other compounds (indole, skatole, and VFAs) were higher in some of the treated samples. As cases in point, the emissions of indole and skatole were higher in the SBP plus CaO_2 treatment at 48 h (Fig. 3c and d) and the VFA emissions were about 10-fold higher in the SBP plus H_2O_2 treatment at 48 h (Fig. 3e). Thus, the question still arises as to the relative importance of these increases in indole, skatole, and/or VFA emissions relative to the decrease in 4-methylphenol emissions. The odor activity value (OAV) analyses provided insight into the overall odor contributions of the different compounds.

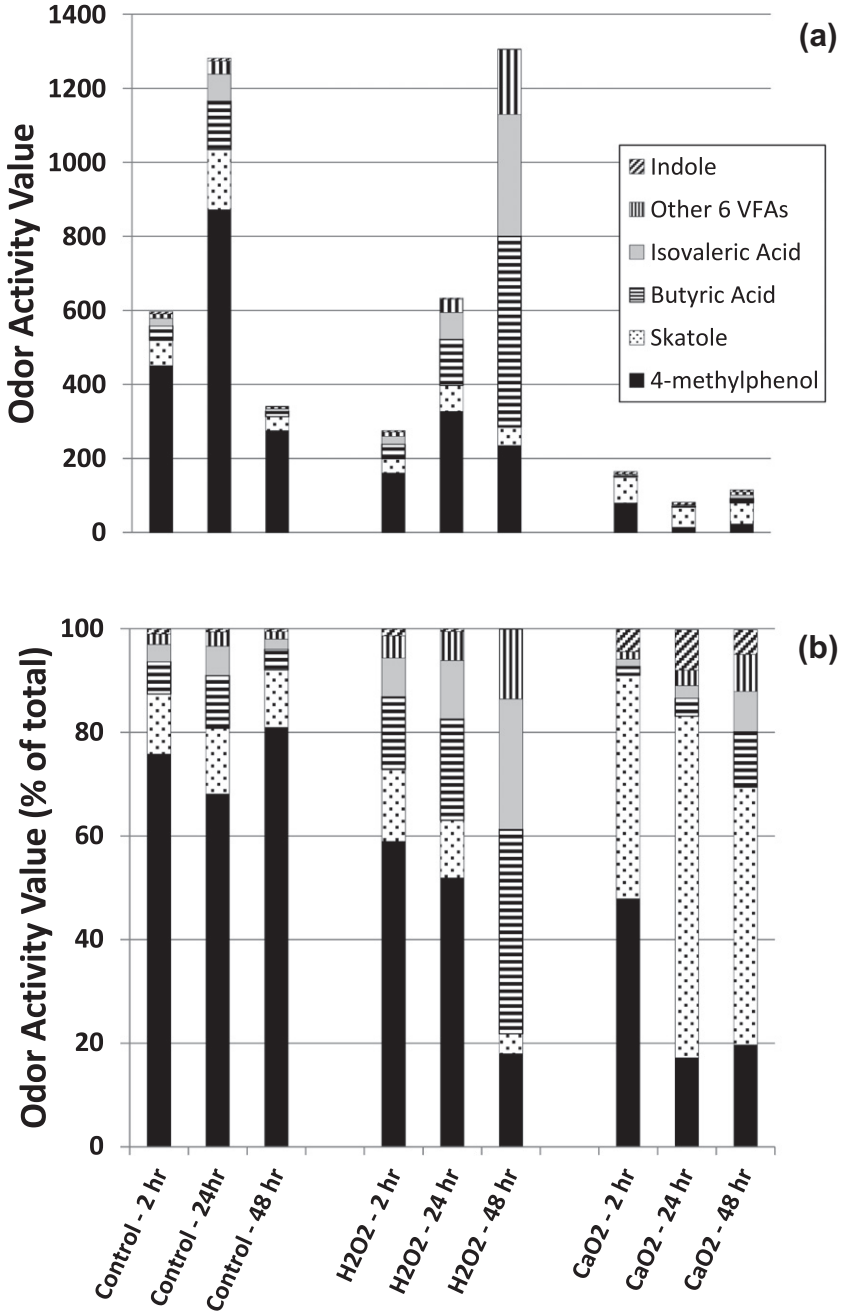

Fig. 4. A comparison of odor activity values (OAV) for TRT 1 (control, no additives), TRT 2 (SBP plus H_2O_2), TRT 3 (SBP plus CaO_2), showing the calculated total OAVs (top) and the relative OAV as a percentage of the summed OAVs of all compounds (bottom).

The geometric mean odor thresholds of indole, skatole, and 4-methylphenol were of similar magnitude, with values of 2.1, 0.48, and 1.3 $\mu g\ m^{-3}$, respectively (Parker et al., in press) and the odor threshold for the sum of eight VFAs was 869 $\mu g\ m^{-3}$. As presented in Fig. 4, 4-methylphenol by itself accounted for 68–81% of the summed OAVs for the control. This OAV compares favorably to the 80% reported by Parker et al. (in press) for land-applied swine manure. Similarly, Feilberg et al. (2010) reported that 4-methylphenol had the highest concentrations above land applied swine manure slurry (175–300 ppb for 4-methylphenol compared to 0–30 ppb for other odorous compounds). Other compounds contributing considerably to the summed OAVs of the control were skatole (11–13%), butyric acid (4–10%), and isovaleric acid (2–6%).

The OAV of the control at 2 h was 597, compared to 275 (54% reduction) for the H_2O_2 treatment, and 165 (72% reduction) for the CaO_2 treatment while after 48 h, there was a 283% increase in OAV for the H_2O_2 treatment and a 66% reduction for CaO_2. Note that even though there was a reduction in 4-methylphenol in the H_2O_2 treatment, the increase in VFA emissions caused an overall increase in OAV.

The actual increase in indole emission rate was 0.53 $\mu g\ m^{-2}\ min^{-1}$ after 48 h (Fig. 3c) which equates to an increase in its contribution to OAV of 3.8-fold. However, the decrease in 4-methylphenol of 22.0 $\mu g\ m^{-2}\ min^{-1}$ (Fig. 3b) equates to a decrease in OAV of 253-fold. Similarly, the increase in OAV for skatole due to its increase in flux of 0.62 $\mu g\ m^{-2}\ min^{-1}$ (Fig. 3d) was 19.4 times, again much smaller than the 253-fold decrease in OAV for 4-methylphenol. Thus, the reduction in 4-methylphenol flux far outweighed the increased flux for indole and skatole in terms of odor emissions.

Finally, the odor threshold for the sum of eight VFAs of 869 $\mu g\ m^{-3}$ equates to an approximate emission rate of 58 $\mu g\ m^{-2}\ min^{-1}$. The actual increase in VFA emissions with the H_2O_2 treatment was about 873 $\mu g\ m^{-2}\ min^{-1}$, for a 15-fold increase in OAV. Again, compared to the 253-fold decrease in OAV for 4-methylphenol, the overall increase in odor for the VFAs was overshadowed by the greater decrease in OAV for 4-methylphenol.

3.7. Safety and economics

Soybean peroxidase is a fine organic powder, and it should be handled with the same precautions necessary for grain dust, which can be explosive if dispersed or suspended in air and ignited. Therefore, soybean peroxidase should be applied directly to the swine manure such that dispersion into the air is minimized. In addition, oxidizable materials such as calcium peroxide can be ignited by grinding (FMC, 2009). For this reason, calcium peroxide should always be added to the soybean peroxidase after the soybean hulls have been ground or otherwise processed.

A detailed economic analysis was deemed premature until further field experiments have been conducted. Nevertheless, the use of highly purified SBP such as that discussed in Hailu et al. (2010) appears to be uneconomical at this point. Hailu et al. (2010) reported a price of 7.5 USD per million units of enzyme activity for highly purified SBP. At this price, the cost to treat all of the manure from a single pig would exceed 100 USD, which is obviously uneconomical with today's pork prices.

3.8. Opportunities for future research

Despite the poor economics of purified SBP, the use of low purity, industrial grade SBP such as that used in this research, if only applied topically to the manure, would likely be more economical. Future studies are warranted to focus on the topical application of SBP to manure in larger pilot-scale laboratory containers, followed by field studies of SBP application in operating swine barns. In addition, polyethylene glycol (PEG) or other secondary additives as discussed in Wu et al. (1997) could possibly be used to improve treatment performance and economics.

4. Conclusions

For treating swine manure slurry, the optimum SBP application rate was 50 $g\ L^{-1}$ when combined with H_2O_2 or CaO_2 concentrations of 14.6 or 22.1 mM, respectively. The SBP plus H_2O_2 treatment resulted in a corresponding 10-fold increase in VFA emissions. The SBP plus CaO_2 treatment was most effective, decreasing the emission rates of the primary odorant 4-methylphenol by 98% and 92% after 24 and 48 h, respectively, with corresponding decreases in summed OAVs of 94% and 68%. The overall results of our SBPP approach suggest that SBP plus CaO_2 could be a promising treatment option for odor reduction from swine manure.

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